Effects of Benzimidazole Compounds for Dutch Elm Disease Control on Wood Surrounding Elm Injection Sites

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ABSTRACT

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Two benzimidazole compounds, thiabendazole hypophosphite (Arbotect 20-S) and methyl 2-benzimidazolecarbamate phosphate (Lignasan BLP), were injected into elms (*Ulmus americana*) at ground level or in root flares to control Dutch elm disease caused by *Ceratocystis ulmi*. Trees harvested 9 mo after injection had significantly more discoloration around Arbotect 20-S injection sites than around Lignasan BLP or water injection sites and noninjected wounds. Scanning electron microscopy demonstrated the lack of tyloses in vessels immediately surrounding the Arbotect 20-S and Lignasan BLP injection wounds. Benzimidazoles appear to alter the normal defense response of the tree and change the sequence of microorganisms that colonize elm wood after wounding. Procedures to minimize internal injury are presented.

Dutch elm disease (DED), caused by *Ceratocystis ulmi* (Buism.) C. Moreau, is the most destructive shade tree disease in North America. All species of elm occurring naturally in the United States are susceptible, but the disease is most damaging to the American elm (*Ulmus americana* L.), formerly the most widely planted shade tree in the United States. An intensive sanitation program is the best way to control DED. Chemical control of DED is possible with the injection of solubilized benzimidazoles (6,7,13,15). Methyl 2-benzimidazolecarbamate phosphate (MBC-H_3PO_4) (Lignasan BLP) and thiabendazole hypophosphite (Arbotect 20-S) are two solubilized benzimidazoles currently used to control DED.

A major concern when systemic fungicides are injected into high-value elm trees to control DED is the amount of internal injury that results from the injection wounds. The process of boring holes into the trunks of trees has long been known to cause discoloration and decay (8). Shigo and Campana (11) observed varying degrees of compartmentalization of the discolored and decayed wood associated with injection holes; decay was associated with 14 of 25 wounded trees.

The internal defect associated with injection wounds appears to be caused by the benzimidazoles injected into the tree as well as by the injection wound (9). Andersen et al (1,2) found that the extent of discoloration and tissue necrosis is the result of chemical toxicity. Using two injection sites (trunk injection and exposed root flare injection), we attempted to ascertain the effects of these chemicals on internal compartmentalization and the sequence of microorganisms that colonize xylem after wounding.

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MATERIALS AND METHODS

Sixteen healthy American elms, eight located in North Oaks, Ramsey County, MN, and the other eight at the University of Minnesota Landscape Arboretum near Chaska, Carver County, MN, were selected. Trees ranged from 20.4 to 43.1 cm (mean 28.6 cm) in diameter 1.4 m above the ground (dbh).

Four holes, 0.67 cm in diameter and 3–5 cm deep, were drilled perpendicular to the injection surface with a high-torque electric drill equipped with a Greenley number 177 spur bit on the north, east, south, and west sides of each tree either in exposed root flares or at ground level. Each wound received one of four treatments: injection with Arbotect 20-S, 3.0 g/L, 1.86 L/cm dbh, in accordance with the Minnesota label (US EPA SLN No. MN 80-0012); injection with Lignasan BLP, 0.825 g/L, 1.86 L/cm dbh; injection with water at 1.86 L/cm dbh; or wounded but no injection. The sixteen trees were randomly assigned to four sets, those injected in exposed root flares (sod and soil were removed from around the base of the tree to a depth of 20–30 cm) and felled after 6 mo or after 9 mo, and those injected at ground level (injections made as close to the ground as possible) and felled after 6 mo or after 9 mo. Treatments were randomly assigned to the first tree of each set and then systematically rotated around each subsequent tree to avoid any effects from the orientation of the wound.

The trees were injected with a low-pressure apparatus that used a 9.5-L hand-pump sprayer tank as a reservoir. Polyvinyl tubing connected each sprayer tank to a brass tee with a tapered nipple. After the holes were drilled, pressure was added to the system, all air within the system was evacuated, and the tee was tapped into the hole with a wooden mallet. Pressure was held between 0.70 and 1.05 kg/cm^2 during the injection process. The elms were injected between 23 August and 7 September 1979.

Eight trees (four injected at ground level and four injected in root flares) were felled 6 mo after injection and the remaining eight trees after 9 mo. Bolts containing the injection wound and the associated discolored xylem were debarked and brought into the laboratory.

These bolts were then cut longitudinally through the center of the injection wound with a handsaw to yield two billets. One billet from each pair was cut in cross section through the injection site. The radial, vertical, and lateral extent of wound-initiated discoloration was measured, and volume of the discolored columns was determined from the volumetric equation for a pyramid. Means of these measurements were tested for significance with the Studentized Newman-Keuls sequential Q-test (14).

Samples of clear and discolored tissues were removed aseptically from the billet immediately above the injection site and prepared for observation with a scanning electron microscope. Specimens were mounted on aluminum stubs and air-dried in a sealed bell jar over lithium chloride crystals at 25 C. The specimens were coated with gold and examined with a Philips SEM 500 scanning electron microscope.

The second billets were used for isolation of microorganisms. Chips (10 × 3 × 3 mm) were aseptically removed from

the discolored tissue, discolored-clear tissue interface, and clear tissue beyond the discolored column with a sterile, V-shaped wood gouge. Chips from each tissue type were placed into each of four types of agar medium so that each chip touched the bottom of the petri dish. The four types of agar used were 1.5% malt-yeast extract agar (15 g of malt) extract, 2 g of yeast extract, and 15 g of agar per liter); 1.5% malt-yeast extract agar acidified with 4 ml of concentrated 85% lactic acid; a selective malt agar medium for basidiomycetes (15 g of malt extract, 2 g of yeast extract, 15 g of agar, 60 mg of benomyl [50% WP], 10 mg of streptomycin sulfate, and 4 ml of concentrated 85% lactic acid per liter) (3); and Difco actinomycete isolation agar.

Chips used for actinomycete isolation were heated in sterile, distilled water at 65 C for 1 hr before being placed on the actinomycete isolation agar (4). Malt agar plates were incubated at 25 C, and the actinomycete isolation agar plates were incubated at 30 C. All microorganisms emerging from the 4,606 chips were subcultured for identification.

RESULTS

The lateral extent of discoloration in wounds injected with Arbotect 20-S was significantly greater ($P = 0.05$) than that in noninjected wounds after 6 mo (Table 1) and was greater than that in all other treatments after 9 mo (Table 1). The vertical extent of discoloration did not differ significantly among treatments after 6 and 9 mo (Table 1). Volumes of discoloration, obtained from radial, vertical, and lateral measurements, did not differ significantly among treatments after 6 mo (Table 1), but after 9 mo, the Arbotect 20-S treatment mean was significantly greater ($P = 0.05$) than all other treatment means.

Volumes of discoloration were grouped by injection site and treatment to determine the effect of injection site (root flare vs ground level) on internal defect. No significant difference was found between root flare means and ground level means. These means have been combined in Table 1.

Discolored xylem immediately above the injection wound was observed with scanning electron microscopy for all

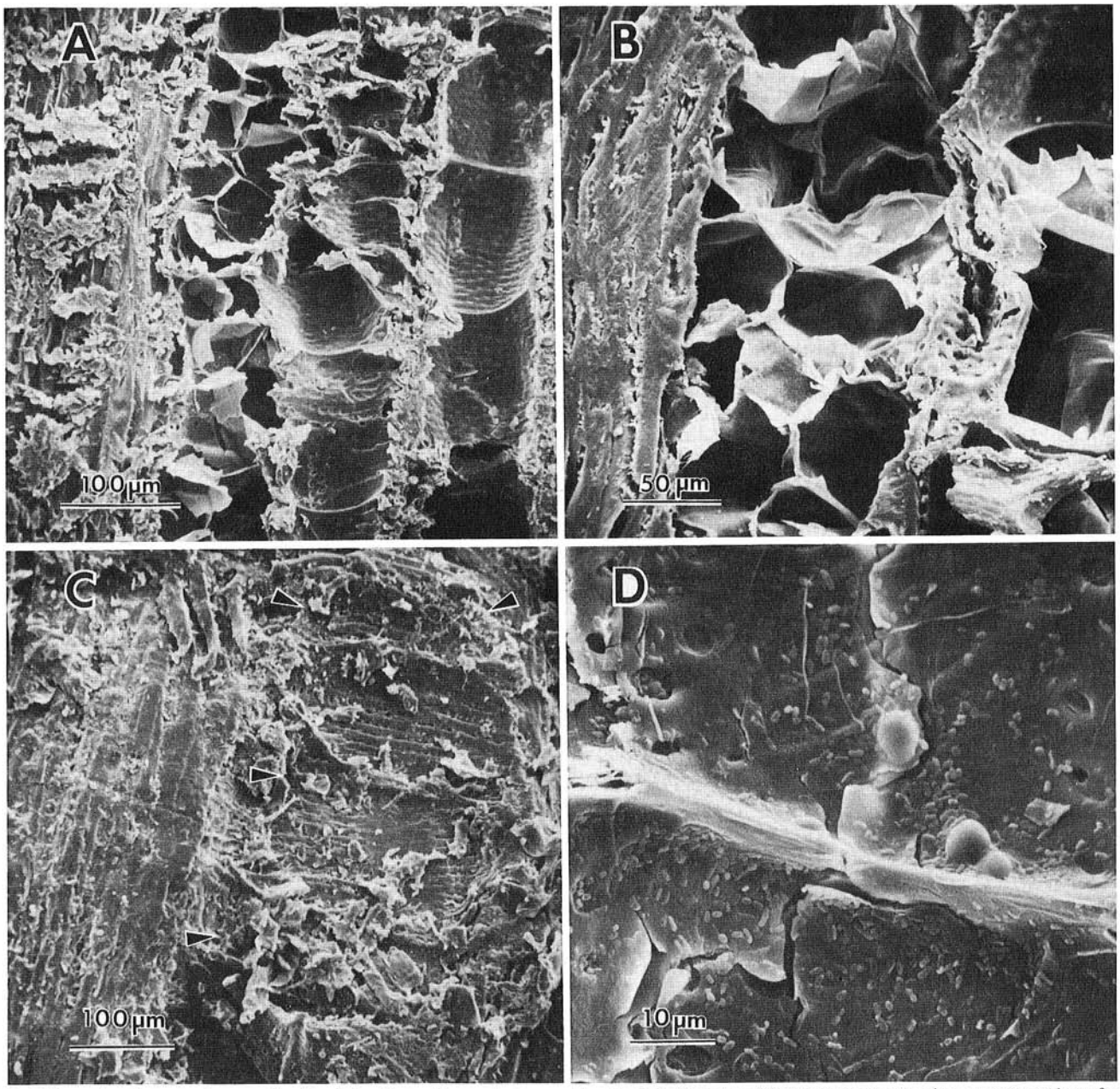

Fig. 1. Scanning electron micrographs of radial sections of wounds surrounding elm injection sites: **(A)** Xylem vessels showing numerous tyloses from noninjected wound. **(B)** Tyloses in vessel from a water-injected wound. **(C)** Xylem vessel from wound treated with thiabendazole hypophosphite (Arbotect 20-S), showing an accumulation of unidentified substances throughout. No tyloses were present. Arrowheads outline vessel. **(D)** Xylem vessel from wound treated with methyl 2-benzimidazolecarbamate phosphate (Lignasan BLP) with bacteria lining the walls.

treatments. Numerous tyloses were observed to have occluded the xylem vessels in noninjected (Fig. 1A) and water-injected wounds (Fig. 1B). Tyloses were infrequently observed from similar xylem tissues above wounds injected with Arbotect 20-S (Fig. 1C) and Lignasan BLP (Fig. 1D). Bacteria were frequently seen in xylem vessels of the Lignasan BLP and Arbotect 20-S treatment wounds (Fig. 1D). An unidentified substance was repeatedly observed coating the vessel walls of the wounds treated with Lignasan BLP and Arbotect 20-S (Figs. 1C and 1D, respectively).

Fewer microorganisms were isolated from the root flare wounds than from the ground level wounds (Table 2). Wounds injected with Arbotect 20-S or Lignasan BLP had fewer hyphomycetes than the noninjected control wounds. More chips yielded basidiomycetes from wounds treated with Arbotect 20-S than from wounds given other treatments. A detailed account of the genera of microorganisms isolated from this study is available from the authors upon request.

DISCUSSION

The significantly greater volume and lateral extent of defect associated with Arbotect 20-S treatment wounds may have important implications. Because preventive treatments for DED control require reinjection in later years, the extent of lateral defect will limit the area for injection sites. The root flares, where injection wounds should be made for good chemical distribution, are the regions of main support for the tree. Decay developing in this area would make the tree highly hazardous as well as bring about its loss as a valuable landscape tree. Increased internal defects around injection wounds treated with Arbotect 20-S have also been reported by Andersen et al (1) and Nishijima (9).

Hyphomycetes are known to be very sensitive to benzimidazole compounds (5). The reduction in numbers of hyphomycetes isolated from injected wounds is probably caused by these fungicides. Benzimidazole compounds do not inhibit basidiomycetes. Although the numbers of basidiomycetes were low, they appear to be associated with the Arbotect 20-S treatment wounds.

The scanning electron micrographs revealed an unidentified material commonly coating the vessel walls of the Lignasan BLP and Arbotect 20-S treatment wounds. Roy et al (10) presented evidence that poor fungicide distribution and premature stoppage of flow in the xylem during the injection with Lignasan are the results of precipitation of MBC. The unidentified material observed in the scanning electron micrographs may be a precipitate of the fungicides' active ingredients.

The effects of Arbotect 20-S in elms appear to resemble those of paraformaldehyde in sugar maples. Paraformaldehyde has been used in sugar maple tapholes to increase and prolong sap yield. Paraformaldehyde alters the tree's normal defense systems by delaying or preventing the formation of vessel plugs and by killing xylem cells so rapidly that the phenolic compounds may not be formed (12,16,17). If the phenolic compounds are not formed, then the usual groups of wound-infecting pioneer microorganisms do not colonize the tissue, and wood-decaying basidiomycetes easily and rapidly invade the wood.

Arbotect 20-S injections appear to alter the normal defense response of the tree and the successional patterns of microorganisms in a manner similar to that caused by paraformaldehyde. Paraformaldehyde is no longer recommended for use in sugar maple tapholes because of the resulting internal injury (17). However, internal injury associated with the injection of systemic fungicides can be minimized with careful and conscientious injection techniques.

Injection should be recommended for high-value elms that have not been exposed to possible root-graft transmission and that are located in areas where intensive sanitation is practiced. Trees should be injected in healthy, exposed root flares, and injection should be done in the late spring and early summer, after full leaf expansion, to ensure maximum chemical distribution and rapid wound closure. Injection wounds should be 10–15 cm (4–6 in.) apart so that the columns of discolored wood do not coalesce and for maximum chemical distribution. Injection holes should be shallow, because the outer xylem ring is the most active conducting tissue in ring-porous species such as elm (18). Shallow injection holes also minimize the amount of discolored wood and prevent the wound from penetrating the central core of discolored wood or wetwood. The holes should be clean-edged and small to ensure rapid wound closure.

Discoloration and decay may be of secondary importance when a tree is almost certain to die from DED in the case of therapeutic treatments, but the same does not hold for preventive treatments. Repeated preventive treatments may protect and save elms from DED, but such treatments cause extensive internal injury (11) and could directly or indirectly kill trees by reducing their resistance to decay (9).

Table 1. Lateral, vertical, and volume measurements of discoloration in elm wounds 6 and 9 mo after treatment

	Dimension of discoloration[w,x]					
	Lateral (mm)		Vertical (cm)		Volume (cm^3)	
Treatment	6 mo	9 mo	6 mo	9 mo	6 mo	9 mo
None	8.00 a	12.12 a	7.72 a	15.61 a	10.00 a	25.07 a
Water	9.12 ab	13.50 a	11.48 a	14.46 a	14.77 a	26.26 a
Lignasan BLP[y]	9.12 ab	13.75 a	16.10 a	15.48 a	22.19 a	27.66 a
Arbotect 20-S[z]	11.38 b	28.62 b	15.72 a	22.10 a	27.07 a	96.01 b

[w] Measurements are means of eight wounds.
[x] Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to the Newman-Keuls test.
[y] Methyl 2-benzimidazolecarbamate phosphate.
[z] Thiabendazole hypophosphite.

Table 2. Number of microorganisms isolated from 4,606 chips from treated elm wounds

	Microorganism					
Treatment Injection site	Bacteria	Hyphomycetes	Yeasts	Actinomycetes	Basidiomycetes	No growth
None						
Ground level	146	216	19	14	3	274
Root flare	182	72	23	8	0	307
Total	328	288	42	22	3	581
Water						
Ground level	156	109	44	4	0	300
Root flare	194	40	23	4	1	319
Total	350	149	67	8	1	619
Lignasan BLP[a]						
Ground level	149	117	15	6	2	314
Root flare	184	25	27	6	0	338
Total	333	142	42	12	2	652
Arbotect 20-S[b]						
Ground level	156	95	54	8	21	298
Root flare	165	20	11	5	1	274
Total	321	115	65	13	22	672

[a] Methyl 2-benzimidazolecarbamate phosphate, 0.825 g/L, 1.86 L/cm dbh.
[b] Thiabendazole hypophosphite, 3.0 g/L, 1.86 L/cm dbh.

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